Changes in Interstitial Metabolic Parameters during Hemorrhagic Shock

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ABSTRACT

Decompensation in hemorrhagic shock is the critical stage after which resuscitative efforts may prove futile. One mechanism for decompensation may be potassium-mediated vasodilation and/or loss of cardiac contractility, and thus a method of measuring interstitial potassium may be a crucial part of future metabolic monitoring efforts. Anesthetized rats underwent controlled hemorrhage to a constant mean arterial pressure of 40 mmHg. Microdialysis probes were implanted in skeletal muscle, vein, and liver for continuous assessment of potassium, glucose, lactate, pyruvate, and glycerol concentrations. Arterial blood samples were drawn at 30-minute intervals, until late (decompensatory) hemorrhagic shock was reached. Potassium concentrations in muscle interstitium were significantly higher in hemorrhaged animals than controls (2.34 times baseline vs. 1.24, $p < 0.05$*), this difference was not reflected in blood values. These data may provide clues into new ways to monitor and treat victims of hemorrhagic shock on the battlefield.*

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1.0 INTRODUCTION

Decompensation is the critical "point of no return" in hemorrhagic shock. It is defined as sustained hypoperfusion leading to irreversible cardiovascular collapse (1), and is manifested clinically as the loss of ability to mount a hemodynamic response to aggressive fluid resuscitation. It is generally stated that once decompensation is reached, further resuscitative efforts are futile (1). Several chemical substances have been proposed as markers for tracking the evolution of hemorrhagic shock and impending collapse; these include pH, lactate, pyruvate, glucose, and base excess. An important military goal of metabolic monitoring would thus be identifying the most critical factors heralding decompensation in battlefield casualties. In addition to providing the ability to assess the stage or severity of hemorrhagic shock, such monitoring may establish prognosis and/or determine the adequacy of therapeutic interventions.

The relationship between serum concentrations—which are commonly measured experimentally and in controlled clinical settings—and the corresponding concentrations in the tissue interstitium has not been explored systematically. This has important consequences for metabolic monitoring because it may be faster and more practical on the battlefield to sample from a transcutaneous or intramuscular sensor than to attempt intravascular sampling. Additionally, the interstitial level may be more representative of the end-organ milieu—and thus a better indicator of underlying pathophysiology. Further information is thus needed about the correlation (or lack thereof) between interstitial and intravascular concentration with regards to monitoring for decompensation.

It is possible that these substances serve not just as indicators, but also as actual mediators of the pathogenesis of decompensation. Specifically, muscle interstitial potassium has been shown to rise out of proportion to intravascular levels during hemorrhage (2;3). These changes are consistent with an effective loss of Na^+-K^+ ATPase (NKA) activity. Hyperkalemia may be a mechanism for vascular smooth muscle hyper-polarization and vasodilatation observed at the decompensatory stage (4;5), which can be mediated by the inwardly rectifying potassium Kir channels or by NKA (6;7).

Our objectives were to revisit the issue of interstitial changes in potassium and other metabolic substances (glucose, lactate, pyruvate, and glycerol) in the pathogenesis of hemorrhagic shock. In addition, we wished to examine how tissue-specific interstitial concentrations correlated with intravascular values. Our hypothesis was that interstitial hyperkalemia in skeletal muscle may herald the onset of decompensation.

2.0 METHODS

2.1 Animals

Male Wistar rats (n = 14, Charles River Laboratories, Wilmington, MA) were quarantined for ten days in a temperature- and light- controlled environment. Animals had *ad libitum* access to rodent chow (Nestlé Purina, St. Louis, MO) and water. After quarantine, animals were weighed for five to seven days prior to use in order to document continued weight gain (5 g/day). Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations related to animals, and experiments involving animals adheres to principles stated in the Guide to the Care and Use of Laboratory Animals, National Research Council.

2.2 Surgical Preparation

On the day of experimentation, the rats were initially anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally. Once a surgical plane of anesthesia had been attained, the rats were shaved and the skin cleaned. A tracheostomy was performed using PE 240 tubing (Clay Adams, Inc., Parsippany, NJ). Under aseptic conditions, catheters (PE50, Clay-Adams) were placed in the following locations: left femoral artery, left carotid artery, left femoral vein, and caudal right quadrant of the peritoneum. The left femoral artery catheter was attached to a continuous blood pressure monitor (BPA, Micromed, Louisville, KY). The left carotid artery catheter was attached to a computer controlled peristaltic pump (Model 720, Instech Laboratories, Inc., Plymouth Meeting, MA) that emptied into a heparinized reservoir placed on a balance (PB303S with RS232 port, Mettler, Inc., Toledo, OH). The left femoral vein catheter was used for venous access as needed.

The peritoneal catheter was used to provide a continuous infusion of sodium pentobarbital (10% in normal saline at 0.06 µl/min/g) administered to maintain the anesthetic plane. Additional pentobarbital was administered as a 0.05-0.1 ml intraperitoneal bolus as needed to maintain loss of digital and corneal reflexes.

Microdialysis probes (CMA/20, CMA/Microdialysis, North Chelmsford, MA) were placed in a branch of the right femoral vein, in the quadriceps major of the right leg, and in the liver through an abdominal incision.

A rectal thermistor temperature probe was inserted and the core temperature was maintained at 37 ^{o}C by a homeothermic blanket (Harvard Apparatus, South Natick, MA) and heating lamp. Each animal was anticoagulated with porcine heparin (1 IU/g i.v. to a maximum 350 IU) prior to the start of the stabilization period plus an additional 100 IU i.v. 60 min later.

2.3 Hemorrhage Protocol

The WRAIR Hemorrhagic Shock Data Acquisition (HSDAQ) Program, an interactive program written in LabVIEW (National Instruments, Austin, TX) controlled the hemorrhage. This program monitored arterial blood pressure and the weight of the shed blood volume (SBV) removed from the animal. It controlled the peristaltic pump to maintain blood pressure at the desired level. Arterial pressures (systolic, diastolic, and mean), heart rate, and shed blood volume were monitored continuously and recorded every 5 seconds by the program.

Controlled hemorrhage was performed following our established protocol. The HSDAQ program was started and the animal was monitored initially for a 20-minute stabilization period after completion of the surgery. After the control period, the program commenced hemorrhage by withdrawing blood from the carotid artery into the reservoir. Mean arterial pressure (MAP) was linearly dropped to 40 mm Hg over a 15 minute time period, then maintained at that value for the duration of the experiment by the additional withdrawal or return of shed blood to the animal (with program providing feedback control). In the initial (compensatory) phase of hemorrhage, blood had to be continuously withdrawn from the animal to maintain the desired MAP. After a period, however, the shed blood had to be returned to the animal from the reservoir to maintain this pressure. The point where this transition occurs is the start of decompensation, and thereafter the phase of shock is designated by the amount of blood that has been returned to the animal, expressed as a percentage of the peak SBV (8). The experiment continued until return of 50% of the peak SBV, at which time the animal was euthanized.

Control animals underwent the same surgical preparation as the bled animals but the peristaltic pump was never activated. The duration of the control experiments were matched to that of the preceding hemorrhage experiment.

2.4 Sample Collection and Analysis

Arterial blood samples were collected from the left femoral line at $t = -17$ min from the start of the bleed (during the stabilization period), at t = 43 min, and subsequently at thirty-minute intervals. Microhematocrit was measured in a centrifuge (Model TRIAC, Clay Adams). Arterial blood gases and potassium were measured using an i-STAT portable clinical analyzer (i-STAT Corp., East Windsor, NJ).

Microdialysis was performed continuously during hemorrhage by perfusing the implanted probes with normal saline containing 4.5 mM RbCl. The perfusate rate was 1 µl/min and it was collected in 15-µl fractions by a fractional collector (CMA/142, CMA Microdialysis).

Potassium concentrations in the microdialysis samples were measured using the internal standard technique (9) to correct for lack of 100% recovery in the probes: the fractional loss of rubidium from the perfusate was assumed equal to the fractional uptake of potassium into the perfusate from the surrounding tissue. 10-µl aliquots of the collected fractions were diluted 1:1000 in 10 ml of 2% HNO_3. Potassium and rubidium concentrations were then measured using inductively-coupled plasma mass spectrometry (Elan 6000, Perkin-Elmer, Norwalk, CT).

Concentrations of glucose, lactate, pyruvate, and glycerol were measured in the remainder of the microdialysis samples using a commercial analyzer (CMA 600). Internal standards were not used for these analytes, thus the concentrations obtained are underestimates.

2.5 Statistical Analysis

Statistical significance between groups was determined using two-tailed Student's test for comparing means of independent measurements. Significance was defined as a p value < 0.05.

3.0 RESULTS

Table 1 shows the baseline data for the animals. There was no significant difference in animal weight or starting MAP between groups. There were no statistical differences between groups for any of the baseline analyte values in any of the tissues. The mean peak SBV for the hemorrhage group was 5.6 ± 0.6 ml and the mean time to peak SBV was 42.6 ± 3.1 min.

Figure 1 shows hemodynamic data (mean arterial pressure and shed blood volume) versus time from start of hemorrhage. The very small error bars for the hemorrhage MAP vs. time demonstrate how the HSDAQ program reproducibly maintains the hemorrhaged animals at the desired target pressure.

Shown in Figures 2-3 are arterial and microdialysis results for $[K^+]$ and various other metabolic parameters.. To emphasize the changes with time, all values are shown as ratios to baseline.

	Control (n = 7)	Hemorrhage (n = 7)
Weight (g)	422±18	423±20
MAP (mmHg)	86.4±6.3	88.5±4.8
HR (bpm)	388±12	373±10
pH	7.42±0.01	7.42±0.01
BE	3.0±0.5	2.3±0.4
Hct	52.6±0.6	51.6±1.1
PSBV (ml)	-------	5.6±0.6
Time to PSBV (min)	-------	42.6±3.1

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	Arterial		μD-Vein		μD-Muscle		μD-Liver	
	C	H	C	H	C	H	C	H
$[K^+]_0$ (mM)	4.5 ±0.5	4.8 ±0.7	4.6 ±0.4	4.7 ±0.6	5.2 ±0.8	6.3 ±0.9	5.1 ±0.8	5.0 ±1.1
$[Glucose]_0$ (mg/dL)	215 ±21	172 ±25	99 ±10	140 ±52	81 ±13	52 ±11	196 ±43	238 ±60
$[Lactate]_0$ (mM)	2.4 ±0.4	1.8 ±0.3	1.4 ±0.3	1.3 ±0.2	1.3 ±0.6	1.2 ±0.1	2.4 ±0.6	3.3 ±0.8
$[Pyruvate]_0$ (μM)	135 ±21	115 ±12	114 ±25	85 ±27	46 ±14	39 ±19	13 ±2	29 ±10
$[L/P]_0$	18 ±2	17 ±1	15 ±2	26 ±13	28 ±7	56 ±21	248 ±134	135 ±76
$[Glycerol]_0$ (mM)	1422 ±209	1219 ±261	262 ±81	230 ±42	222 ±127	181 ±50	397 ±117	435 ±155

Table 1: Baseline values for animals. All values shown ± one S.E.M. (*Left*) MAP = mean arterial pressure, HR = heart rate, BE = base excess, Hct = hematocrit, PSBV = peak shed blood volume, L/P = lactate/pyruvate ratio. (*Right*) Baseline measurements of metabolic parameters from arterial samples and microdialysis (μD) probes, performed at t = –17 min from start of bleed. $p > 0.05$ for all values (C vs. H).

Potassium (Figure 2, top panels): Interstitial $[K^+]$ was higher in muscle at peak SBV than controls (ratio = 2.33 vs. 1.24 times baseline, $p < 0.05$); this was not reflected in vein or liver. These results are consistent with previous studies (2;3). After peak SBV, average muscle $[K^+]$ declines versus time due to dropout of animals reaching experimental end.

Glucose (Figure 2, middle and bottom panels): Arterial glucose ratios were higher (2.68 vs. 1.06, $p < 0.05$) at peak SBV than controls, and then decreased. This hyperglycemia during hemorrhagic shock and subsequent fall in glucose levels during decompensation has been previously described (10). There were similar trends in venous and muscle samples, but they did not reach significance. In liver, no trend versus time for either group was appreciated.

Lactate (Figure 3, top panel): Values in all tissues were higher in hemorrhaged animals, but only reached significance in venous samples at t = 28 (ratio = 3.05 vs. 1.42) and 43 (6.91 vs. 1.67) min. In muscle similar differences were seen (ratios = 2.8 vs. 0.8 at 28 min and 4.9 vs. 0.8 at 43 min) but hemorrhage vs. control differences were not significant due to small n = 4 from analytical problems. In liver the difference at t = 28 min just fell short of statistical significance (2.74 vs. 1.32, $p = 0.068$). The smaller relative increase in muscle and liver is consistent with previous results and has been used to argue that these tissues are lactate consumers in hemorrhage (11).

Pyruvate (Figure 3, second panels): Venous samples tended to increase with time in both hemorrhage and control groups without statistically significant difference. Samples from muscle and liver in both groups showed no clear change with time.

L/P ratio (Figure 3, third panels): Trended upward with time in control liver samples and all hemorrhaged samples without significant statistical difference.

Glycerol (Figure 3, bottom panels): Levels in all tissues tended to increase in both hemorrhage and control animals, without significant difference between groups.

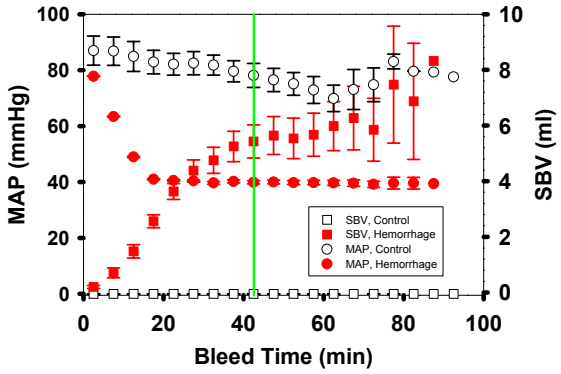

Figure 1: Mean arterial pressure (MAP) and shed blood volume (SBV) vs. time from start of bleed. Error bars designate ± one S.E.M. The vertical line delineates the average time to peak shed blood volume for hemorrhaged animals.

4.0 SUMMARY/CONCLUSIONS

Interstitial [K^+] in skeletal muscle during hemorrhage appears to correlate with the onset of decompensation, while intravascular [K^+] does not. Maximal hyperglycemia also correlates with peak SBV. Muscle and liver glucose may be similarly correlated, although the magnitude of the change appears to be less. The rise in venous lactate levels from microdialysis also corresponded with peak SBV. Changes in tissue lactate had similar trends but did not reach statistical significance due to small numbers. Interstitial measures of potassium, lactate, and/or glucose may prove to be of diagnostic and prognostic significance in hemorrhagic shock.

Interstitial hyperkalemia during hemorrhage has previously been demonstrated with ion sensitive electrodes (2) and ion sensitive field effect transistors (3). Furthermore, x-ray microanalysis studies have shown that intracellularly, potassium decreases and sodium increases during hemorrhage (12). These changes are consistent with a derangement of Na^+-K^+ ATPase (NKA) activity. The nature of this derangement is unknown. Possible mechanisms include loss of available NKA or substrate, presence of an *in vivo* NKA inhibitor, or an uncoupling of NKA activity to potassium transport. Given the possible significance of hyperkalemia as an etiology for decompensation, future study in this area is warranted.

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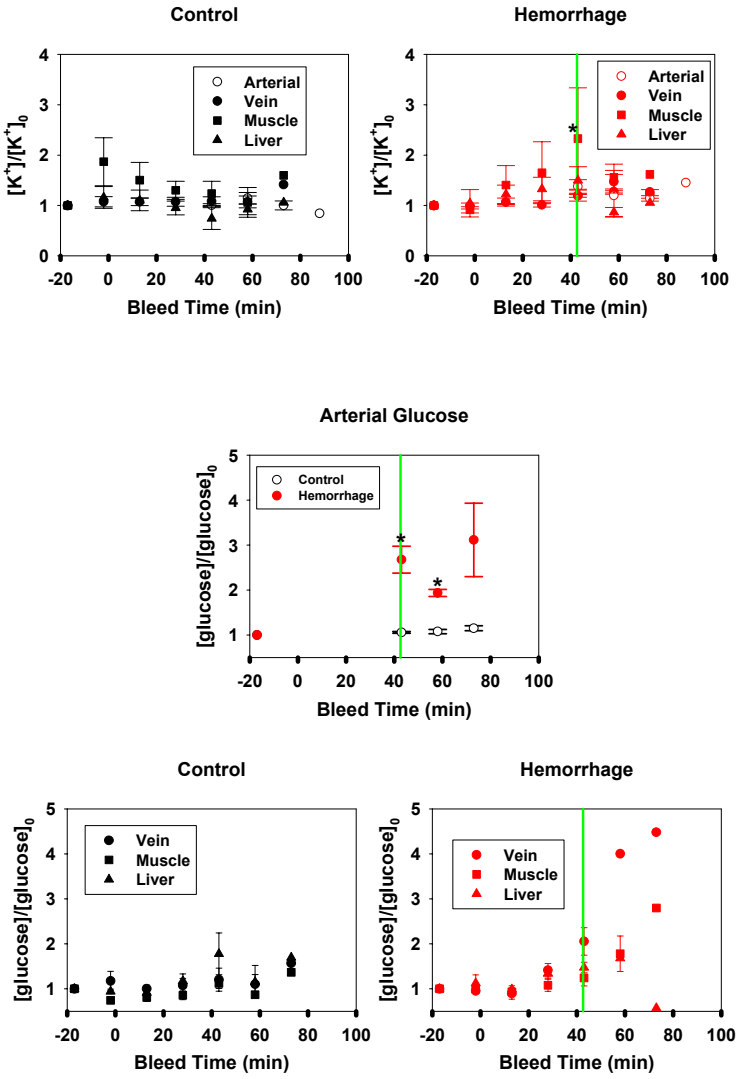

Figure 2: Relative changes in potassium and glucose. Error bars designate ± one S.E.M. Vertical lines in hemorrhage plots delineate average time to peak shed blood volume. Top panels: Potassium in arterial and microdialysis (vein, muscle, liver) samples. Center panel: Arterial glucose. Bottom panels: Microdialysis glucose from tissues. *$p < 0.05$ for hemorrhage vs. control.

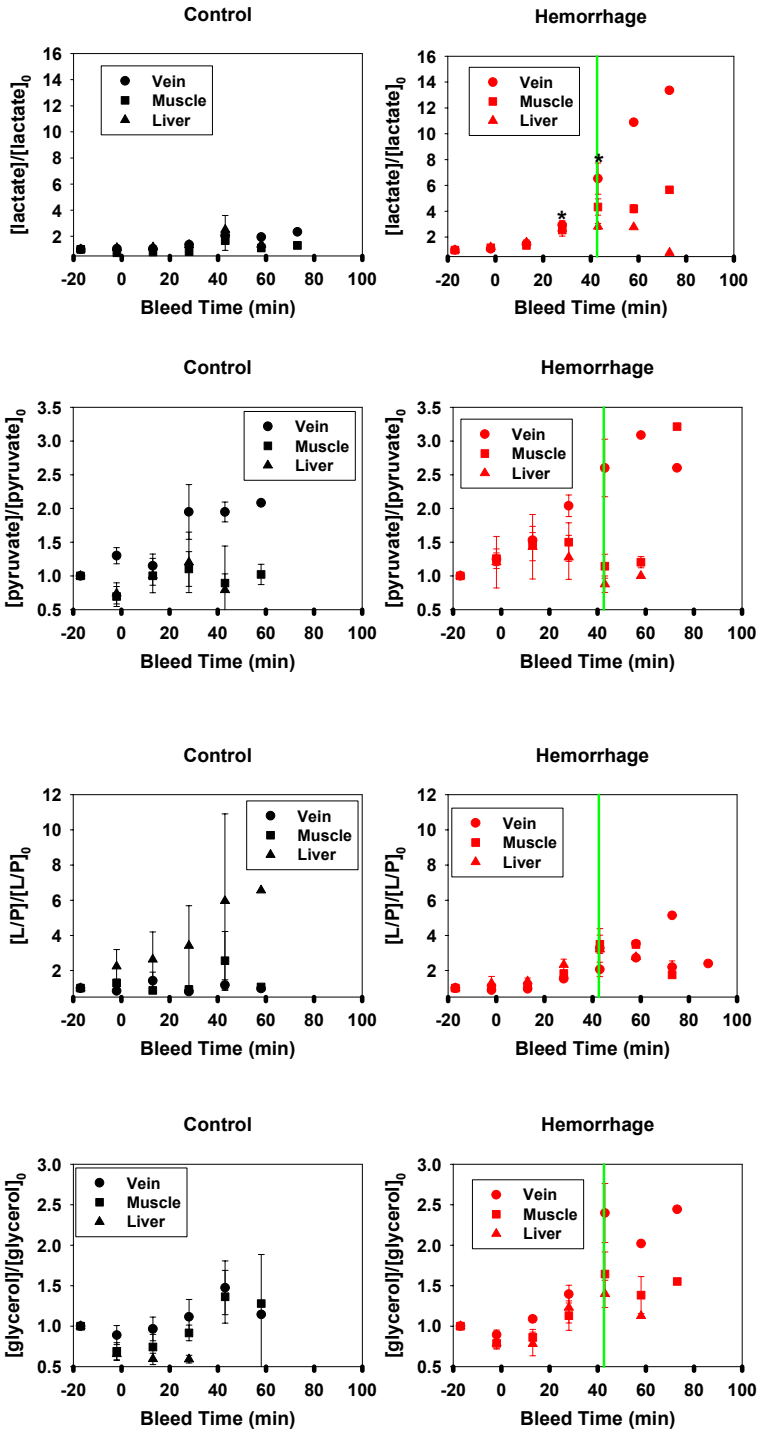

Figure 3: Relative changes in lactate, pyruvate, L/P ratio, and glycerol. Error bars designate ± one S.E.M. Vertical lines in hemorrhage plots delineate average time to peak shed blood volume. *$p < 0.05$ for hemorrhage vs. control.

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